Vaginitis: current microbiologic and clinical concepts

Leslie V.H. Hill,* MD, MSc
Juan A. Embil,†‡§ MD, PhD, FRCPC, FACTM

Infectious vaginitis occurs when the normal vaginal flora is disrupted; it may arise when saprophytes overwhelm the host immune response, when pathogenic organisms are introduced into the vagina or when changes in substrate allow an imbalance of microorganisms to develop. Examples of these types of vaginitis include the presence of chronic fungal infection in women with an inadequate cellular immune response to the yeast, the introduction of trichomonads into vaginal epithelium that has a sufficient supply of glycogen, and the alteration in bacterial flora, normally dominated by *Lactobacillus* spp., and its metabolites that is characteristic of "nonspecific vaginitis". The authors review microbiologic and clinical aspects of the fungal, protozoal and bacterial infections, including the interactions of bacteria thought to produce nonspecific vaginitis, that are now recognized as causing vaginitis. Other causes of vaginitis are also discussed.

L'infection vaginale résulte d'une altération de la flore: soit que des saprophytes prennent le dessus contre les défenses immunitaires, soit que des pathogènes soient introduits de l'extérieur, soit que la modification du milieu amène un déséquilibre microbien. On donne comme exemples la vaginite mycosique de la femme dépourvue d'une immunité cellulaire suffisante contre les moisissures, la pénétration de *Trichomonas* dans une muqueuse vaginale suffisamment pourvue de glycogène, et la perturbation de la flore bactérienne (où dominent normalement les lactobacilles) et de ses métabolites. Ce dernier état de choses est typique de la vaginite dite non-spécifique. On passe en revue la clinique et la microbiologie des vaginites à moisissures, à protozoaires et à bactéries; on décrit les interactions microbiennes qui sont en cause dans la vaginite non-spécifique. On explique aussi les autres causes de vaginite.

From the departments of *Obstetrics and Gynecology, †Microbiology, ‡Pediatrics and §Community Health and Epidemiology, Dalhousie University, Halifax

Reprint requests to: Dr. Juan A. Embil, Infection and Immunology Research Laboratory, Izaak Walton Killam Hospital for Children, 5850 University Ave., Halifax, NS B3J 3G9

The normal microflora of the vagina is composed of different types of organisms whose relative numbers fluctuate according to the quantity and form of available substrates. Thus, the concept of a stable normal vaginal flora composed of *Lactobacillus* spp., with an average pH of 4, is idealized. For the purposes of this article we will assume that a dynamic normal vaginal flora exists[1] and that infectious vaginitis arises when saprophytes overwhelm the host response, when pathogenic organisms are introduced into the vagina or when an imbalance in the normal flora develops.

Yeasts

History

The first recorded reference to thrush infections (yeast on mucous membranes) was made in 400 BC, when Hippocrates described white patches associated with debilitating illnesses. Vaginal fungi were first associated with vaginitis by Wilkinson, in 1849, and "*Monilia* vulvovaginitis" was first described by Plass and colleagues, in 1931.[2] In 1954 *Candida* was officially accepted as the genus name for *Monilia albicans*.[2]

Prevalence and morbidity

The prevalence of the different types of vaginitis varies widely from one study to another, possibly because of differences in study populations or in the microbiologic methods used. There is also wide variation in the reported prevalence of genital symptoms among women with the various types of vaginitis.

Jones and Warnock[3] found that only 30% of women with vaginal candidiasis were symptomatic, while Carroll and associates[4] reported that *C. albicans* was associated with "vulvovaginal mor-

bidity" in 84% of patients. One of us (L.V.H.H.) has found that 24% of 266 women attending a university student health centre harboured vaginal yeasts, but only 74% of these patients had clinical signs of vaginitis.[5]

Prevalence in other sites

The role of an "anal reservoir" in vaginal candidiasis has been controversial. When genital tract infection persists despite adequate antifungal therapy, the gastrointestinal tract is commonly cited as the focus from which reinfection arises. In a prospective 4-month study no evidence that the vagina was infected from the rectum or vice versa was obtained.[6] Our work also does not support the notion that an anal reservoir is necessary for the occurrence of vaginal yeasts: of 65 women in whom vaginal yeasts were cultured only 40% concurrently harboured anal yeasts.[5]

The urine is not normally a source of fungal invasion of the vulva and vagina. Of women with yeast vaginitis 54% also had yeasts in their urine.[7] In all cases the same species were involved, and when the vaginal infections were eliminated the yeast spontaneously disappeared from the urine. Urine from the husbands of women with chronic yeast vaginitis gave positive results when cultured for yeast in 45% of cases,[8] which suggests a male genital reservoir as a possible source of reinfection.

Positive results of yeast cultures have been obtained from the coronal sulcus, urine, and prostatic and seminal secretions. Most cases of male genital yeast infections are caused by transmission from an infected sexual partner.[9] This suggests that yeast eradication must be undertaken simultaneously in both partners to prevent reinfection. The presence or absence of a prepuce does not seem to affect the carriage rate of yeasts on the penis: circumcised and uncircumcised men yielded yeast at the same rate in a study by Davidson.[10]

Pathogenesis

Pathogenicity of mycelial (filamentous) and yeast (budding) forms of vaginal fungi is disputed, though both are isolated from fungal lesions. Fungal invasion has been documented in deep-seated vaginitis by electron micrography, which showed single epithelial cells containing many intracellular budding yeast cells.[11] Yeasts were also shown penetrating living vaginal epithelial cells, including those in deeper epithelial layers. Filamentous cells may be able to press through cell membranes, whereas budding cells are easily diverted.[12]

The relative pathogenicity of *C. albicans* is disputed because it does not always fulfil Koch's postulates. *C. albicans* can exist as a saprophyte on mucous membranes, and previous damage to the vagina may be required for it to produce vaginitis. Genital yeast infections may do this by combining with bacterial or viral infection, by disrupting the mucous membranes or even by worsening the symptoms already being produced by other factors.[6] Cutaneous candidiasis cannot be produced experimentally unless an occlusive, sticky dressing is placed over the inoculum[13] because *C. albicans* is rarely able to establish itself on intact dry skin. Mucin rather than saline suspension appears to increase the virulence of intraperitoneal *C. albicans* infections in animal studies.[13] Because they increase local moisture, panty hose and nylon underwear may predispose to yeast vaginitis.

Lactobacilli are the predominant vaginal organisms in women without vaginitis or with yeast vaginitis. A natural balance may exist between lactobacilli and *C. albicans* in which the fungus provides growth factors for the bacterium and vice versa. When *C. albicans* is present, *Lactobacillus acidophilus* is capable of growing on a medium that is adequate for fungal growth but that lacks several vitamins essential for the growth of lactobacilli.[14] The latter split glycogen, produced by vaginal epithelial cells, into glucose and maltose; *C. albicans* does not proliferate when glycogen is the sole carbohydrate source, but when lactobacilli are added the released simple sugars act as a growth medium for the yeast.[15]

An increase in glycogen production is commonly believed to result in an increased possibility of yeast vaginitis. Such an increase occurs in diabetes mellitus, nondiabetic glycosuria and pregnancy. However, most women with chronic recurrent fungal vaginitis have normal results in glucose tolerance tests, which suggests that recurrent vulvovaginal candidiasis is unlikely to be the only manifestation of occult diabetes mellitus.[16]

Since humoral immunity is of secondary importance in fungal infections compared with cell-mediated immunity, the increased prevalence of symptomatic yeast vaginitis in the third trimester of pregnancy may be due to reduced cell-mediated immunity in the presence of normal humoral antibodies[17] rather than to elevated glycogen production. Despite an otherwise normal immune response, nonpregnant women with recurrent vaginal yeast infections appear to produce *Candida*-specific suppressor lymphocytes that block the cellular immune response to this organism.[18] Thus, an exogenous source of inoculum may be less important than decreased host resistance in the development of symptomatic disease.

Oral contraceptives increase estrogen and progesterone levels and therefore increase glycogen production, but there have been conflicting reports as to whether they predispose to yeast infection.[17] Administration of systemic adrenal steroids predisposes to the development of systemic fungal infections, but there is little evidence that it predisposes to fungal vulvovaginitis. Schnell[15] compared growth curves of yeast from 30 vaginal secretion specimens "from women with varying hormonal situations of very different endogenous

or exogenous origins" and concluded that a direct influence of sex hormones and their metabolites on yeast growth could largely be excluded. Because corticosteroids stabilize lysosomal membranes, they may predispose to fungal overgrowth; however, the indirect effects of hormones on yeast growth have yet to be well established.

Antibiotics, particularly those with broad-spectrum coverage, are a well documented cause of yeast vaginitis. Recently, metronidazole has also been implicated: when a 2-day course of the drug was given, yeast vaginitis developed in 6% to 8% of patients, but when it was given for 7 days, yeast vaginitis developed in 26% of those treated.[17]

C. albicans is widely recognized as a pathogenic yeast; however, in symptomatic cases other yeast species, including *Torulopsis glabrata, C. tropicalis, C. stellatoidea, C. krusei, C. parapsilosis, C. pseudotropicalis* and *C. guilliermondi*, may be found in the vagina.[19] The percentage of species found other than *C. albicans* is thought to be higher in unselected patients than in those with symptoms of vaginitis;[20] we have been unable to document this finding. Although 76% of 50 women with *C. albicans* infection and only 50% of 15 women harbouring yeast species other than *C. albicans* had clinical evidence of vaginitis, this difference was not statistically significant.[5]

Treatment

Isolated and sporadic episodes of yeast vaginitis are easily treated, but recurrent yeast vaginitis poses a much greater challenge. Local therapy may be ineffective in recurrent infections because it clears only the superficial infection, while the fungi may penetrate into deeper layers.[21] Many antifungal agents are available, but nystatin, synthetic imidazoles and gentian violet are among the most effective.[22] Nystatin binds to sterols in fungal membranes, which causes leakage of intracellular components. The drug is not well absorbed and thus is frequently used as an antifungal agent in the gastrointestinal tract. Synthetic imidazoles are believed to inhibit fungal cell wall synthesis and are widely reported to be as effective as or more effective than nystatin. Since miconazole nitrate is absorbed into the blood through mucous membranes in small amounts, nystatin is the recommended antifungal agent in pregnancy. Other synthetic imidazoles include clotrimazole and econazole nitrate. Gentian violet has traditionally been used in yeast vulvovaginitis and is thought to act by interfering with fungal enzymes. Because the dye has been known to cause severe allergic reactions in some patients, nystatin, which is nonsensitizing, is better used in sporadic episodes.[23]

Women with diabetes who have chronic yeast vaginitis frequently suffer from vulvovaginitis produced by both chronic trauma and chronic infection. This form of neurodermatitis can be eliminated by corticosteroid therapy given concurrently with topical antifungal agents.[23]

Gardner[23] offers several recommendations for the treatment of chronic recurrent infections: (a) longer therapy, not interrupted during menstruation; (b) use of an intravaginal candidicide nightly for a few days before each menstrual period as a prophylactic measure; (c) use of an intravaginal candidicide during and for several days after any course of antibiotic therapy; (d) discontinuation of oral contraceptives; and (e) application of nystatin ointment or miconazole cream to the patient's preputial folds and to her sexual partner's penis twice daily for 10 days. If all of these measures fail, nystatin, 500 000 to 1 million U taken orally four times daily for 7 to 14 days, may be tried (although the role of an anal reservoir in vaginal infections is controversial). Long-term topical therapy with nystatin vaginal tablets may be the only way to prevent symptoms in some patients.

Boric acid powder has recently been recommended for the treatment of vulvovaginal candidiasis. Van Slyke and colleagues[24] compared treatment with boric acid powder, 600 mg administered intravaginally 14 times a day, with treatment with nystatin, 100 000 U administered intravaginally 14 times a day. The cure rates with boric acid powder were 92% 7 to 10 days after treatment and 72% 30 days after treatment, whereas the corresponding rates with nystatin were 64% and 50%. Serum boron analysis showed slight vaginal boron uptake (less than 1 μg/mL), well below the level at which toxic effects occur.

Mead[22] recommends the following modifications in treating yeast vulvovaginitis in infants and young children. Nystatin cream is applied to the child's vulva and perineum four times daily or after each diaper change. In mild infections 1 mL of a 0.5% aqueous solution of gentian violet is instilled into the vagina with a sterile eyedropper each night for 10 nights. In more severe infections 1 mL of nystatin suspension (100 000 U/mL) is instilled into the vagina three times daily for 10 days, and 1 mL of the same suspension is administered orally four times a day.

Culture rather than clinical resolution of symptoms and signs provides the best test of cure in both infants and adults.

Complications

Three main types of complications arise from yeast vulvovaginitis: fungal balanoposthitis in male sexual partners, antepartum or intrapartum neonatal contamination and disseminated fungal infection.[22] Amniotic infection with yeasts is extremely rare, but once it develops, multiple organ and umbilical cord involvement follows. Infection can occur across intact membranes, may result in prematurity or spontaneous abortion, and is often associated with an intrauterine device (IUD).[25] Intrapartum infection may cause thrush, or coloniza-

tion of the oral cavity, in neonates. Screening and treatment before delivery of pregnant women with positive results of vaginal yeast cultures easily prevents the development of neonatal thrush.

Trichomonads

History

Trichomonas vaginalis was first described in the human vagina by Alfred Donne, in 1836.[26] By 1894 several workers had described trichomonads in the male urinary tract.[26] The notion that *T. vaginalis* may cause vaginitis was not proposed until 1916.[27]

Prevalence and morbidity

Trichomoniasis is well documented as a sexually transmitted disease (STD). Patients with several sexual partners are at increased risk for infection, and, because of this, the prevalence varies widely according to the population studied. More than half of prostitutes harbour *T. vaginalis*,[28] while 10% of patients at an STD clinic in Halifax[29] and 5% of patients at a venereology clinic in London[30] were found to have cultural evidence of vaginal trichomoniasis. Trichomoniasis has been found among 3% to 15% of asymptomatic women attending gynecology clinics and among 20% to 50% of symptomatic patients attending STD clinics.[28] In our own centre trichomoniasis was found among 13 of 128 patients (10%) attending gynecology, STD and prenatal clinics; when questioned, 11 of the 13 (85%) complained of excessive vaginal discharge, malodour or irritation.[29]

Prevalence in other sites

T. vaginalis is the only known human pathogen of the genus *Trichomonas* and can be isolated from urethral specimens from both sexes as well as from vaginal secretions. Gardner[23] was able to recover trichomonads from the urethra of most male sexual contacts of women with trichomoniasis and suggested that less than 20% of men are symptomatic. Trichomoniasis is generally believed to be a self-limited infection in males when the lower urethra is colonized, but this has not been confirmed. Trichomonads can occasionally be isolated from the prostate gland, seminal vesicles and epididymis as well as the urethra. Semen samples are preferred for detecting trichomonads in the male since they yield more positive results of culture than do urine samples.[26]

Pathogenesis

T. vaginalis vaginitis was produced in human volunteers by inoculation with "bacteria-free" cultures of *T. vaginalis* in 1940.[27] The protozoans exert a vigorous mechanical motion against human cells in culture,[31] and these motions have been suggested to be largely responsible for their cytotoxic action. The virulence of *T. vaginalis* has been found to vary widely in strains studied so far.

Because well glycogenated squamous epithelium enhances growth of *T. vaginalis*,[32] infections in prepubescent girls and postmenopausal women, in whom estrogen and progesterone levels are decreased, are often asymptomatic. An elevated vaginal pH encourages the growth of trichomonads, but there is disagreement as to whether the elevated pH is due to the trichomoniasis or vice versa.[27,32,33] A strong association between *Gardnerella vaginalis*, anaerobic bacteria, vaginal pH above 4.5 and the presence of amines in vaginal fluid has been found not only in nonspecific vaginitis but also in trichomonal and gonococcal infection.[34] Trichomoniasis commonly coexists with gonococcal infections and is associated with absence of yeast infections (which usually occur at a lower pH).[35]

Treatment

Orally administered metronidazole is effective against *T. vaginalis* and nonspecific (bacterial) vaginal infections. When urogenital trichomoniasis is detected both sexual partners should be simultaneously treated with a single 2-g dose of metronidazole. When given orally the drug reaches the bladder, urethra, Skene's and Bartholin's glands, seminal vesicles, prostate and endocervix, while topical administration, which is common, does not always eliminate trichomonads in the urethra or periurethral structures. The single 2-g dose treatment has a short-term cure rate of 85%, and unreliable patients can take it under observation.[36]

Metronidazole should be used during the second half of pregnancy only if patients remain symptomatic after attempts at vaginal acidification.[36] Controversy exists over the mutagenic and carcinogenic potential of metronidazole, which crosses the placenta and is excreted in breast milk, although there is no evidence that it causes human fetal or neonatal damage when ingested ante or post partum.[37] The recommended dosage of metronidazole after 20 weeks' gestation is 500 mg twice daily for 3 to 5 days.[27]

Complications

The main difficulty with trichomoniasis is not in complications from the infection but in persistent or recurrent disease. In such patients a detailed sexual history is necessary to determine whether there are untreated partners and whether all partners were treated simultaneously.

Trichomoniasis was formerly thought to be primarily a "nuisance" infection with no evidence of fetal or neonatal morbidity. However, culture-proven *T. vaginalis* respiratory tract infection has recently been described in two infants with respiratory tract disease.[38] Forty-one reports of trichomonads in the respiratory tract of adults were recently reviewed.[39] The diagnosis was unexpected and was usually made by means of wet-mount examination of sputum, although pulmonary parenchyma and pleural fluid also yielded the organisms. The finding of pulmonary trichomonal infection in neonates with respiratory tract disease suggests that women with vaginal trichomoniasis and their partners should be treated (although treatment should not be started until after 20 weeks' gestation, given the current controversy regarding toxic effects of metronidazole in pregnancy).

T. vaginalis infection may promote infertility by facilitating movement of microorganisms from the vagina to the fallopian tubes. Trichomonads have been isolated from the pouch of Douglas and the uterine tubes by means of laparoscopy and may therefore act as vectors in the production of polymicrobial pelvic inflammatory disease (PID).[40] Recent evidence suggests that pregnant women with *T. vaginalis* infection are more likely than those without the infection to have premature rupture of the membranes,[41] which further supports the argument for treatment of trichomoniasis in the second half of pregnancy.

Bacteria

History

Vaginitis caused by neither trichomoniasis nor candidiasis has been termed nonspecific vaginitis. In 1955 Gardner and Dukes[42] proposed that any woman whose vaginal pH is greater than 5 and whose vaginal secretions are grey and homogeneous and contain "clue" cells (vaginal epithelial cells disintegrating under an overlying coat of bacteria) most likely has *Haemophilus vaginalis*[43] vaginitis (currently known as *Gardnerella vaginalis* vaginitis). Investigators in 1978[44] noted that vaginal discharges with these characteristics emitted a fishy, amine odour with, and often without, addition of 10% potassium hydroxide. Increased numbers of anaerobic bacteria were also detected in these abnormal secretions. The distinctive odour was found in 1979 to be due to volatilization of polyamines, particularly putrescine and cadaverine, presumably produced by vaginal anaerobic bacteria.[45] This finding set in motion the current controversy about the three possible causes of nonspecific vaginitis: *G. vaginalis*, vaginal anaerobic bacteria or an interaction of the two types of organisms. In women without nonspecific vaginitis *Lactobacillus* predominates in the vaginal flora, although *G. vaginalis* can be found in low concentrations with selective differential media.[46] In women with the condition both anaerobic bacteria and *G. vaginalis* are present in higher numbers.[47] Both the prevalence and numbers of *G. vaginalis*, *Bacteroides* spp. (excluding *B. fragilis*), *Peptococcus* spp. and *Eubacterium* spp. were found to be increased in women with nonspecific vaginitis, while numbers of *Bifidobacterium* spp. and anaerobic *Lactobacillus* spp. were found to be increased and those of facultative *Lactobacillus* spp. decreased in women with the condition.[48]

In 1913 Curtis isolated a motile curved anaerobic bacillus from pathogenic "uterine discharges".[49] Several groups are currently investigating this organism, which is frequently associated with nonspecific vaginitis.[49] A new genus name, *Mobiluncus*, has recently been proposed to describe these curved rods of vaginal origin.[50] Their precise role in the pathogenesis of nonspecific vaginitis remains unclear.

Prevalence and morbidity

Symptoms of vaginitis alone are not reliable indicators of the presence of vaginitis, particularly nonspecific (bacterial) vaginitis. This infection is characterized by a relative lack of inflammatory response[42] compared with that produced by trichomoniasis or candidiasis. The prevalence of bacterial vaginitis varies according to the clinical criteria applied; for this reason, objective clinical signs should be used to make a clinical diagnosis of bacterial vaginitis. Amsel and colleagues[51] proposed that patients be considered to have nonspecific vaginitis if they have vaginal secretions that have a pH greater than 4.5, are homogeneous and of low viscosity, contain clue cells and release an amine odour when mixed with 10% potassium hydroxide.

Although few prevalence studies have strictly adhered to these criteria, the prevalence of nonspecific vaginitis appears to be slightly higher among patients at STD clinics. The condition was found in 21% of patients at a student health clinic in Seattle, Washington,[52] 23% of those at family planning and prenatal clinics in Halifax,[29] 35% of those at a venereology clinic in London,[15] 37% of those at an STD clinic in Halifax[29] and 64% of those at an STD clinic in Boston.[53]

Prevalence in other sites

G. vaginalis and anaerobes are carried by men urethrally or subpreputially.[54,55] *G. vaginalis* has been recovered from 96% of male sexual contacts of women with nonspecific vaginitis, which further supports the notion of sexual transmission.[42] The frequent recurrence of *G. vaginalis* infection in women treated for bacterial vaginitis, even if their partners are treated, adds to the need for further study of sexual transmission.

Pathogenesis

The organisms responsible for nonspecific vaginitis have yet to be completely described. Gardner and Dukes[42] tried to fulfil Koch's postulates by inoculating volunteers with pure cultures of *G. vaginalis* but were more successful when they used secretions from infected patients as the inoculum. This finding suggests that other, presumably unknown, organisms are at least partly responsible for the infection.

Taxonomic studies of *G. vaginalis* have shown its optimum pH to be between 6 and 6.5. It does not grow at a pH less than 4, yet it produces acetic acid as its main end product of fermentation and must, therefore, eventually poison itself when grown in pure culture. Thus, the characteristically elevated vaginal pH in nonspecific vaginitis must be due to metabolites of other microorganisms that presumably enhance growth of *G. vaginalis* by maintaining a more alkaline vaginal pH.[43]

The malodour characteristic of vaginal secretions in women with nonspecific vaginitis is thought to be produced by putrescine and cadaverine. A similar malodour is present in women who use intravaginal collagen sponges for contraception.[56] In sponges extracted immediately after intercourse high levels of putrescine, spermidine and spermine but not cadaverine were detected. Chen and associates[45] suggested that metronidazole-sensitive anaerobic bacteria produce putrescine and cadaverine and that these amines are converted from the nonvolatile salt form to the volatile free-base form when the vaginal pH is elevated by alkaline semen or even by soap.

Nonspecific vaginitis is characterized by a relative scarcity of leukocytes in vaginal secretions.[42] This lack of an inflammatory response is important in distinguishing the condition from other types of vaginal infections and suggests that the term "nonspecific vaginitis" is a misnomer. The term "nonspecific vaginosis" has been suggested to reflect the scarcity of inflammatory cells associated with the discharge.[47] Whether nonspecific vaginitis is to be renamed "clue-cell vaginosis", "anaerobic vaginosis",[57] "bacterial vaginosis"[48] or "vaginal bacteriosis" has yet to be resolved. The abbreviation "NSV" is easily confused with that of nonspecific urethritis (NSU), and this gives further impetus to the search for a new name.

In nonspecific vaginitis levels of organic acid metabolites of the microbial flora (other than *Lactobacillus* and *Streptococcus* spp., both lactate producers) normally found in the vagina are elevated.[52] Gas–liquid chromatographic analysis of organic acids revealed increased levels of succinate, acetate, butyrate and propionate. Predominant organisms included *G. vaginalis* (an acetate producer), *Bacteroides* spp. (succinate producers) and *Peptococcus* spp. (butyrate and acetate producers). The criteria for a presumptive diagnosis of nonspecific vaginitis by gas–liquid chromatographic analysis of vaginal secretions are a ratio of the peak height of succinate (in millimetres) to the peak height of lactate (in millimetres) greater than 0.4; a peak height of acetate of more than 2 mm; or detection of propionate, isobutyrate, butyrate or isovalerate.[53]

The mixed flora of *G. vaginalis* and anaerobes appears to produce the amines and γ-aminobutyric acid characteristically found in vaginal secretions of women with nonspecific vaginitis[45,58] as well as the short-chain organic acids described above.[53]

Amsel and colleagues[51] found that women with nonspecific vaginitis were more likely than women without the condition to be using IUDs (20% v. 6%). Conversely, among women attending family planning clinics in Finland 20% of those using IUDs had discharges characteristic of nonspecific vaginitis, compared with 5% of those not using IUDs.[59] The normal vaginal flora was replaced by the mixture of anaerobes and *G. vaginalis* characteristically found in nonspecific vaginitis; this mixed flora was found in samples from the vagina, endometrium and IUD, which supports arguments that the IUD and its tail facilitate bacterial ascent from the vagina into the endometrial cavity. No correlations between nonspecific vaginitis and other forms of birth control have been reported so far.

Treatment

Metronidazole, 500 mg given orally twice daily for 7 days, is effective in nonspecific vaginitis[44] and has been the most commonly recommended form of therapy. More recently, in a multicentre Norwegian study metronidazole, 2 g given orally as single doses on days 1 and 3, was found to give a cure rate of 94% 4 weeks from the start of treatment.[60] This group also observed that the number of *C. albicans* isolations was significantly higher in patients who received metronidazole for 5 or 7 days, which suggests that shorter treatment is indicated.

Metronidazole has therapeutic efficacy in nonspecific vaginitis but comparative in-vitro insensitivity against *G. vaginalis*. This suggests either that the in-vitro situation does not reflect the in-vivo one or that other pathogens are involved. The hydroxy metabolite of metronidazole has excellent in-vitro activity against *G. vaginalis*.[61] Prolonged metronidazole use has been reported to cause toxic effects to the central nervous system.[62] Sulfonamide cream, doxycycline, ampicillin and erythromycin are ineffective in nonspecific vaginitis.[44,63]

Complications

G. vaginalis septicemia occurs more frequently in obstetrics and gynecology patients than was previously thought; however, most patients recover completely with or without appropriate antimicro-

bial therapy.[64] Urine specimens obtained by means of suprapubic aspiration yielded *G. vaginalis* in 16% of 1000 pregnant women.[65] Neonatal–maternal infections have been reported,[64] and postpartum *G. vaginalis* bacteremia is thought to arise by the organism's gaining access to the bloodstream via an exposed vascular bed rather than as the result of immunosuppression.

In a recent prospective study of the vaginal flora in pregnancy, patients with *Bacteroides* spp. in the vagina during pregnancy were found to be more likely than those without the organism to give birth before 37 weeks' gestation and to have infants weighing less than 2500 g.[41] The same investigators also found preterm rupture of the membranes significantly more often among patients with *Bacteroides* spp. in the vagina and concluded that microbiologic screening in early pregnancy may aid in the assessment of risk for preterm delivery.

Episiotomy wound infection is more likely to develop in women with nonspecific vaginitis. In West Germany nonspecific vaginitis was detected in 5% to 10% of all sexually active women, and one third of women with the condition were reported to have infection of the episiotomy wound after delivery.[66] The investigators suggested that a more aggressive approach to the treatment of asymptomatic nonspecific vaginitis may be warranted.

Diagnosis

Vaginal discharge is a complex mixture of substances from vulvar, sebaceous, sweat, Bartholin's and Skene's glands, exfoliated cells, cervical mucus, secretions of the endometrial cavity and fallopian tubes, and serum transudate of vaginal wall capillaries. These constituents are composed of simple and complex carbohydrates, fatty acids, electrolytes and a complex microflora containing many different organisms.[67] To diagnose vaginitis a physician must distinguish physiologic vaginal secretions from pathological vaginal discharge. This requires both physical examination and rapid laboratory testing.

The extent to which women are affected by vaginal symptoms varies,[56] and complaints of increased vaginal discharge, dysuria and dyspareunia are well known to be nonspecific. In diagnosing vaginitis the physician must examine the vulva and perineum, the vaginal walls and the cervix. Because symptoms are unreliable in predicting which type of vaginitis is present,[68] clinical signs are better used in the diagnosis; diagnosis by telephone is discouraged.

First, an assessment of the macroscopic appearance of vaginal and cervical secretions must be done to distinguish vaginitis from cervicitis. If a purulent discharge is present on the ectocervix after the cervical os is wiped with a cotton-tipped swab, a presumptive diagnosis of cervicitis can be made.[36] Introital discharge is suggestive of cervicitis if grossly purulent, of nonspecific vaginitis if not purulent and of trichomoniasis if of either type. The presence of vulvar erythema, edema or fissures suggests yeast vaginitis. Normal vaginal secretions as well as those of women with yeast vaginitis are characteristically white, thick and floccular. Normal secretions usually pool in the posterior fornix, while those in women with candidiasis adhere to the vaginal walls and may have the classic "cottage-cheese" appearance. Secretions of women with trichomoniasis or nonspecific vaginitis are often present at the introitus, are thin and homogeneous, and adhere to the vaginal walls. Secretions of women with nonspecific vaginitis are characteristically grey, while those in women with trichomoniasis are more yellow-grey.[69]

Following macroscopic assessment of secretions, a drop of the discharge is placed on commercial pH paper. The vaginal pH is normally less than 4.5; if the pH is elevated trichomoniasis or nonspecific vaginitis should be suspected. Cervical mucus, blood and amniotic fluid all falsely elevate the pH.

A drop of 10% potassium hydroxide is then mixed with a drop of secretions. In women with trichomoniasis or nonspecific vaginitis this produces a fishy odour as a result of diamine volatilization.[58,70] The slide can be saved for identification of fungal cells after the squamous cells are destroyed by the potassium hydroxide.

Direct microscopic examination of secretions is commonly used for the presumptive diagnosis of vaginitis while awaiting culture results; however, false-negative and false-positive results frequently occur. Yeasts are identified microscopically with greater accuracy in symptomatic patients (50%) than in asymptomatic patients (15%) with positive results of fungal cultures.[15] This may reflect either higher cell numbers when signs and symptoms are present or increased clinician persistence when fungal infections are suspected. False-positive results are frequently due to over-reading debris on the slide, poor transport or culture methods, or the presence of saprophytic vaginal fungi. *Saccharomyces* and *Cryptococcus* spp., two saprophytic yeast genera, occur as single cells only, never as filaments.

T. vaginalis is a motile protozoan with four flagella that can sometimes be detected with light microscopy. The organisms are approximately the same size as leukocytes and may be difficult to distinguish in wet-mount preparations unless actively motile. More than 10 leukocytes per high power field is correlated with increased risk of *T. vaginalis* infection.[28]

Clue cells are commonly found in nonspecific vaginitis. *G. vaginalis* often predominates in wet-mount preparations, where the organisms often form free-floating clumps or "rafts".[30] With Gram's stain *G. vaginalis* organisms can be identified as pleomorphic gram-variable bacilli.[43]

Culture gives the most accurate diagnosis of candidiasis or trichomoniasis. More accurate re-

sults are obtained if the patient has not douched or used vaginal suppositories for at least 48 hours before the specimens are collected and if the speculum is not lubricated. In contrast, the accurate diagnosis of nonspecific vaginitis is based on the presence of at least three of the four clinical criteria proposed by Amsel and colleagues[51] (vaginal secretions that have a pH greater than 4.5, are thin and homogeneous, contain clue cells and release an amine odour when mixed with 10% potassium hydroxide); the diagnosis can be made without microbial culture or biochemical laboratory tests. The choice of which cultures to order in the diagnosis of nonspecific vaginitis depends on whether the infection is thought to be caused by anaerobes, *G. vaginalis* or microaerophilic motile curved bacilli.[50] For research purposes the detection of characteristic organic acids in secretions by means of gas–liquid chromatography is useful in diagnosing nonspecific vaginitis,[52] as is the detection of diamines in vaginal fluid with thin-layer chromatography.[58]

Colposcopic and Papanicolaou smear changes can sometimes be seen in cases of trichomoniasis, but Papanicolaou smear findings of trichomoniasis should be confirmed with culture since the test gives a high rate of false-positive and false-negative results.[27] Fewer than 10% of women with *T. vaginalis* infection have the classic "strawberry" cervix or vagina.[23] Subepithelial erythema can often be found on colposcopic examination in cases of trichomoniasis and represents diffuse or patchy blood vessel dilation and proliferation in the surface epithelium and submucosa.[27]

Other causes

Noninfectious causes

Noninfectious causes of vulvovaginitis include foreign bodies, chemicals, atrophic vaginitis and neurodermatitis. These causes will not be discussed in detail but are mentioned since they constitute an important part of the differential diagnosis of vulvovaginal inflammation.

Cervicitis

Cervicitis caused by herpes simplex virus (HSV) type 1 or 2, *Neisseria gonorrhoeae* or *Chlamydia trachomatis* can present symptomatically as vaginal discharge. Both *N. gonorrhoeae* and *C. trachomatis* are endocervical organisms, growing on columnar epithelium. These organisms are passed to neonatal epithelium during birth. Silver nitrate prophylaxis effectively prevents neonatal conjunctivitis due to gonococci but does not prevent chlamydial conjunctivitis or other chlamydial infections, such as neonatal pneumonitis and otitis media. Both *N. gonorrhoeae* and *C. trachomatis* are capable of causing urethritis, proctitis, cervicitis, salpingitis and perihepatitis as well as neonatal conjunctivitis. Untreated infections in males may give rise to chronic prostatitis, epididymitis or even urethral stricture. The sequela of untreated infections in females frequently is PID.

C. trachomatis has been isolated from 11% of women attending the Prenatal Clinic at Grace Maternity Hospital, Halifax.[71] Chlamydial infections are more common than gonococcal infections in many populations. At the University of Washington Prenatal Clinic *C. trachomatis* was present in 5% to 10% of all women ante partum, whereas only 1% had gonorrhea; in Sweden it is estimated that up to 60% of cases of salpingitis are caused by chlamydial infection, while only 10% to 20% of all cases of PID are now due to gonorrhea.[72]

HSV types 1 and 2 exist in a latent state in the host's dorsal spinal ganglia and may travel down a nerve root to the skin, where they form vesicles. First cases usually present with both local and systemic signs: vesicles or ulcers on the vulva or cervix, and fever, photophobia, malaise and myalgia. Vaginal discharge is caused by cervicitis; urethral discharge is due to urethritis. The lesions are painful and may take 3 to 4 weeks to completely resolve in first episodes.

HSV may be isolated from the cervix in both symptomatic and asymptomatic patients. In Halifax the virus was isolated from 5.6% and 1.7% of consecutive patients at STD and prenatal clinics respectively.[73]

Complications of genital herpes include viral meningitis (a benign disease that rarely progresses to viral encephalitis), herpes pharyngitis and the development of vesicles on other body sites, believed to occur by autoinoculation.[74] The management of genital herpes in pregnancy is controversial. If the birth canal is infected vaginal delivery should be avoided to prevent intrapartum transmission of the virus. If no evidence of genital herpes is observed in early labour — that is, if no lesions are visible and a Papanicolaou test shows no evidence of viral infection — vaginal delivery should be possible.[74]

Group B streptococcal infections

Group B streptococci are carried asymptomatically in the vagina but will be briefly discussed because they cause neonatal septicemia and meningitis. The relation between vaginal carriage and neonatal disease remains unclear. The organisms may appear and be eliminated spontaneously during pregnancy;[75] thus, there is controversy over whether and when to treat the infection. Fetal infection may occur without maternal fever, leukocytosis, uterine tenderness, or premature or prolonged rupture of the membranes. Perinatal asphyxia due to group B streptococcal infection may cause fetal distress in infants of asymptomatic women with intact membranes. Cases of severe perinatal hypoxic–ischemic encephalopathy associ-

ated with intrauterine group B streptococcal infection in infants of women with intact membranes have been reported.[76] Group B streptococci are found significantly more often in women with increased sexual exposure, in those who use IUDs and during the first half of the menstrual cycle. The organisms were isolated from 18% of college women[77] and from 23% of pregnant women screened in an inner-city population.[78] Rates of vertical transmission to neonates appeared to be influenced by density of colonization in the latter group.

Mycoplasmal infections

Although vaginal discharge cannot be directly attributed to *Mycoplasma hominis* and *Ureaplasma urealyticum*, these organisms have been found to be associated with a number of genitourinary tract infections and complications in pregnant women and neonates. Their presence is correlated with increased sexual activity,[79] complaints of vaginal discharge[80] and nonspecific vaginitis.[81] Isolations of genital mycoplasmas have been associated with clinical evidence of intra-amniotic infection,[82] premature and prolonged rupture of the membranes, low birth weight,[83] placental inflammation,[84] low-grade postpartum fever of short duration[85] and spontaneous abortion.[86,87]

Lactobacilli infections

One case of premature labour and chorioamnionitis associated with *Lactobacillus* spp., followed by possible neonatal infection, has been described.[88] Although lactobacilli are not usually considered to be pathogenic, their classification, particularly when the organisms are obtained clinically, is still incomplete. *Lactobacillus* is normally found in the vagina and comprises several species, including *L. acidophilus, L. fermentum, L. mesenteroides, L. casei* and *L. cellobiosus*. These species have been grouped together as Döderlein's bacillus.

Conclusion

Some of the commonly held clinical beliefs about vaginitis that have not been substantiated by investigation include the notion of an anal reservoir in chronic yeast vaginitis, the belief that women with chronic yeast vaginitis are more likely to have abnormal results of glucose tolerance tests, and the sometimes reported association of oral contraceptives with yeast vaginitis. The belief that trichomoniasis and the abnormal vaginal flora that characterizes nonspecific vaginitis are benign conditions in pregnancy is also being questioned. Recent reports of trichomonads in the respiratory tract of newborns with respiratory disease whose mothers had vaginal trichomoniasis, as well as a reported association between *T. vaginalis* and premature rupture of the membranes, suggest that treatment of trichomoniasis in pregnancy may be of clinical value. Similarly, an association between *Bacteroides* spp. and preterm rupture of the membranes, delivery before 37 weeks' gestation and birth weight less than 2500 g has been reported. An increased likelihood of episiotomy wound sepsis in women with nonspecific vaginitis has also been described.

Microbiologic information on vaginal microflora species and their population dynamics, nutritional needs and possible competitive strategies in vaginitis is only beginning to be collected. Further basic research in these areas will help direct clinical studies about the significance of microorganisms in the vagina.

We thank the Dalhousie Medical Research Foundation, for providing the medical research studentship that funded L.V.H.H. during this review.

References

1. Bartlett JG, Polk BF: Bacterial flora of the vagina: quantitative study. *Rev Infect Dis* 1984; 6 (suppl 1): S67–S72
2. Winner HI, Hurley R: *Candida albicans*, J & A Churchill, London, 1964
3. Jones GR, Warnock DW: Diagnosis of *Candida* vulvovaginitis. *J Clin Pathol* 1978; 31: 98–99
4. Carroll CJ, Hurley R, Stanley VC: Criteria for diagnosis of *Candida* vulvovaginitis in pregnant women. *J Obstet Gynaecol Br Commonw* 1973; 80: 258–263
5. Houck LV: *Prevalence of Genital and Nongenital Yeasts in Women with and without Vaginitis*, thesis, U of Wash, Seattle, 1979
6. Davidson F, Mould RF: Recurrent genital candidosis in women and the effect of intermittent prophylactic treatment. *Br J Vener Dis* 1978; 54: 176–183
7. De Sousa HM, Van Uden N: The mode of infection and reinfection in yeast vulvovaginitis. *Am J Obstet Gynecol* 1960; 80: 1096–1100
8. Kistner RW: *Gynecological Principles and Practice*, Year Bk Med, Chicago, 1971: 83–87
9. Masterton G, Sengupta SM, Schofield CBS: Natamycin in genital candidosis in men. *Br J Vener Dis* 1975; 51: 210–212
10. Davidson F: Yeasts and circumcision in the male. *Br J Vener Dis* 1977; 53: 121–122
11. Schnell JD, Voigt WH: Are yeasts in vaginal smears intracellular or extracellular? *Acta Cytol (Baltimore)* 1976; 20: 343–346
12. Odds FC: *Candida and Candidosis*, Univ Park, Baltimore, 1979: 189–208
13. Maibach HI, Kligman AM: The biology of experimental human cutaneous moniliasis (*Candida albicans*). *Arch Dermatol* 1962; 85: 233–257
14. Koser SA, Hodges E, Tribby I et al: Growth of lactobacilli in association with *Candida albicans*. *J Infect Dis* 1960; 106: 60–68
15. Schnell JD: Investigations into the pathoaetiology and diagnosis of vaginal mycoses. *Chemotherapy* 1982; 28 (suppl 1): 14–21
16. Sobel JD: Vulvovaginal candidiasis — what we do and do not know. *Ann Intern Med* 1984; 101: 390–392
17. Fleury FJ: Recurrent *Candida* vulvovaginitis. *Chemotherapy* 1982; 28 (suppl 1): 48–50

18. Witkin SS, Ru Yu I, Ledger WJ: Inhibition of *Candida albicans*-induced lymphocytes and sera from women with recurrent vaginitis. *Am J Obstet Gynecol* 1983; 147: 809–811
19. Hurley R, Leask BGS, Faktor JA et al: Incidence and distribution of yeast species and *Trichomonas vaginalis* in the vagina of pregnant women. *J Obstet Gynaecol Br Commonw* 1973; 80: 252–257
20. Hurley R, Stanley VC, Leask BGS et al: Microflora of the vagina during pregnancy. *Soc Appl Bacteriol Symp Ser* 1974; 3: 155–185
21. Hosen H: Chronic monilial vaginitis [C]. *Ann Allergy* 1971; 29: 499
22. Mead PB: *Candida albicans*. In Monif GRG (ed): *Infectious Diseases in Obstetrics and Gynecology*, 2nd ed, Har-Row, Philadelphia, 1982: 323–345
23. Gardner HL: Infectious vulvovaginitis. Ibid: 515–541
24. Van Slyke KK, Michel VP, Rein MF: Treatment of vulvovaginal candidiasis with boric acid powder. *Am J Obstet Gynecol* 1981; 141: 145–148
25. Delprado WJ, Baird PJ, Russell P: Placental candidiasis: report of three cases with a review of the literature. *Pathology* 1982; 14: 191–195
26. Kupferberg AB: *Trichomonas vaginalis*: nutritional requirements and diagnostic procedures. *Int Rec Med Gen Pract Clin* 1955; 168: 709–717
27. Bard DS: *Trichomonas vaginalis* (urogenital trichomoniasis). In Monif GRG (ed): *Infectious Diseases in Obstetrics and Gynecology*, 2nd ed, Har-Row, Philadelphia, 1982: 286–300
28. McLellan R, Spence MR, Brockman M et al: The clinical diagnosis of trichomoniasis. *Obstet Gynecol* 1982; 60: 30–34
29. Hill LH, Ruparelia H, Embil JA: Nonspecific vaginitis and other genital infections in three clinic populations. *Sex Transm Dis* 1983; 10: 114–118
30. Hill LVH: Anaerobes and *Gardnerella vaginalis* in nonspecific vaginitis. *Genitourin Med* 1985; 61: 114–119
31. Alderete JF, Pearlman E: Pathogenic *Trichomonas vaginalis* cytotoxicity to cell culture monolayers. *Br J Vener Dis* 1984; 60: 99–105
32. Hildebrandt RJ: Trichomoniasis: always with us — but controllable. *Med Times* 1978; 106: 44–48
33. Parsons CL, Lofland S, Mulholland SG: The effect of trichomonal vaginitis on vaginal pH. *J Urol* 1977; 118: 621–622
34. Ison CA, Easmon CSF, Dawson SG et al: Non-volatile fatty acids in the diagnosis of non-specific vaginitis. *J Clin Pathol* 1983; 36: 1367–1370
35. Fouts AC, Kraus SJ: *Trichomonas vaginalis*: reevaluation of its clinical presentation and laboratory diagnosis. *J Infect Dis* 1980; 141: 137–143
36. Eschenbach DA: Vaginal infection. *Clin Obstet Gynecol* 1983; 26: 186–202
37. Beard CM, Noller KL, O'Fallon WM et al: Lack of evidence for cancer due to use of metronidazole. *N Engl J Med* 1979; 301: 519–522
38. McLaren LC, Davis LE, Healy GR et al: Isolation of *Trichomonas vaginalis* from the respiratory tract of infants with respiratory disease. *Pediatrics* 1983; 71: 888–890
39. Osborne PT, Giltman LI, Uthman EO: Trichomonads in the respiratory tract. A case report and literature review. *Acta Cytol (Baltimore)* 1984; 28: 136–138
40. Keith LG, Berger GS, Edelman DA et al: On the causation of pelvic inflammatory disease. *Am J Obstet Gynecol* 1984; 149: 215–224
41. Minkoff H, Grunebaum AN, Schwarz RH et al: Risk factors for prematurity and premature rupture of membranes: a prospective study of the vaginal flora in pregnancy. *Am J Obstet Gynecol* 1984; 150: 965–972
42. Gardner HL, Dukes CD: *Haemophilus vaginalis* vaginitis. A newly defined specific infection previously classified "nonspecific" vaginitis. *Am J Obstet Gynecol* 1955; 69: 962–976
43. Greenwood JR, Pickett MJ: Transfer of *Haemophilus vaginalis* Gardner and Dukes to a new genus *Gardnerella*: *G. vaginalis* (Gardner and Dukes) comb. nov. *Int J Syst Bacteriol* 1980; 30: 170–178
44. Pheifer TA, Forsyth PS, Durfee MA et al: Nonspecific vaginitis. Role of *Haemophilus vaginalis* and treatment with metronidazole. *N Engl J Med* 1978; 298: 1429–1434
45. Chen KCS, Forsyth PS, Buchanan TM et al: Amine content of vaginal fluid from untreated and treated patients with nonspecific vaginitis. *J Clin Invest* 1979; 63: 828–835
46. Totten PA, Amsel R, Hale J et al: Selective differential human blood bilayer media for isolation of *Gardnerella (Haemophilus) vaginalis*. *J Clin Microbiol* 1982; 15: 141–147
47. Holmes KK, Spiegel C, Amsel AR et al: Nonspecific vaginosis. *Scand J Infect Dis [Suppl]* 1981; 26: 110–114
48. Spiegel CA, Davick P, Totten PA et al: *Gardnerella vaginalis* and anaerobic bacteria in the etiology of bacterial (nonspecific) vaginosis. *Scand J Infect Dis [Suppl]* 1983; 40: 41–46
49. Thomason JL, Schreckenberger PC, Spellacy WN et al: Clinical and microbiological characterization of patients with nonspecific vaginosis associated with motile, curved anaerobic rods. *J Infect Dis* 1984; 149: 801–809
50. Spiegel CA, Roberts M: *Mobiluncus* gen nov, *Mobiluncus curtisii* subsp *curtisii* sp nov, *Mobiluncus curtisii* subsp *holmseii* subsp nov, and *Mobiluncus mulieris* sp nov, curved rods from the human vagina. *Int J Syst Bacteriol* 1984; 34: 177–184
51. Amsel R, Totten PA, Spiegel CA et al: Nonspecific vaginitis — diagnostic criteria and microbial and epidemiologic associations. *Am J Med* 1983; 74: 14–22
52. Spiegel CA, Amsel R, Eschenbach D et al: Anaerobic bacteria in nonspecific vaginitis. *N Engl J Med* 1980; 303: 601–607
53. Embree J, Caliando JJ, McCormack WM: Nonspecific vaginitis among women attending a sexually transmitted diseases clinic. *Sex Transm Dis* 1984; 11: 81–84
54. Kinghorn GR, Jones BM, Chowdhury FH et al: Balanoposthitis associated with *Gardnerella vaginalis* infection in men. *Br J Vener Dis* 1982; 58: 127–129
55. Masfari AN, Kinghorn GR, Duerden BI: Anaerobes in genitourinary infections in men. *Br J Vener Dis* 1983; 59: 255–259
56. Huggins GR, Preti G: Diagnostic clues from vaginal odors. *Contemp Ob/Gyn* 1983; 22: 199–220
57. Blackwell A, Barlow D: Clinic diagnosis of anaerobic vaginosis (non-specific vaginitis). A practical guide. *Br J Vener Dis* 1982; 58: 387–393
58. Chen KCS, Amsel R, Eschenbach DA et al: Biochemical diagnosis of vaginitis: determination of diamines in vaginal fluid. *J Infect Dis* 1982; 145: 337–345
59. Kivijarvi A, Jarvinen H, Gronroos M: Microbiology of vaginitis associated with the intrauterine contraceptive device. *Br J Obstet Gynaecol* 1984; 91: 917–923
60. Jerve F, Berdal TB, Bohman P et al: Metronidazole in the treatment of non-specific vaginitis (NSV). *Br J Vener Dis* 1984; 60: 171–174
61. Ralph ED, Amatnieks YE: Relative susceptibilities of *Gardnerella vaginalis* (*Haemophilus vaginalis*), *Neisseria gonorrhoeae*, and *Bacteroides fragilis* to metronidazole and its two major metabolites. *Sex Transm Dis* 1980; 7: 157–160
62. Alvarez RS, Richardson DA, Bent AE et al: Central nervous system toxicity related to prolonged metronidazole therapy. *Am J Obstet Gynecol* 1983; 145: 640–641
63. Durfee MA, Forsyth PS, Hale JA et al: Ineffectiveness of erythromycin for treatment of *Haemophilus vaginalis*-associated vaginitis: possible relationship to acidity of vaginal secretions. *Antimicrob Agents Chemother* 1979; 16: 635–637
64. Reimer LG, Reller LB: *Gardnerella vaginalis* bacteremia: a review of thirty cases. *Obstet Gynecol* 1984; 64: 170–172
65. McFadyen IR, Eykyn SJ: Suprapubic aspiration of urine in pregnancy. *Lancet* 1968; 1: 1112–1114
66. Petersen EE, Pelz K: Anaerobic vaginosis. *Lancet* 1984; 1: 337–338
67. Huggins GR, Preti G: Vaginal odors and secretions. *Clin*

Obstet Gynecol 1981; 24: 355–377
68. McGuire LS, Guzinski GM, Holmes KK: Psychosexual functioning in symptomatic and asymptomatic women with and without signs of vaginitis. *Am J Obstet Gynecol* 1980; 137: 600–603
69. Eschenbach DA: A guide to diagnosis and treatment of vaginal infection. *Contemp Ob/Gyn* 1982; 20: 203–212
70. Sanderson BE, White E, Balson MJ: Amine content of vaginal fluid from patients with trichomoniasis and *Gardnerella* associated non-specific vaginitis. *Br J Vener Dis* 1983; 59: 302–305
71. Embil JA, Pereira LH: Prevalence of *Chlamydia trachomatis* and genital mycoplasmas in asymptomatic women. *Can Med Assoc J* 1985; 133: 34–35
72. Holmes KK: The *Chlamydia* epidemic. *JAMA* 1981; 245: 1718–1723
73. Embil JA, Garner JB, Pereira LH et al: Association of cytomegalovirus and herpes simplex virus infections of the cervix in four clinic populations. *Sex Transm Dis* 1985; 12: 224–228
74. Corey L: The diagnosis and treatment of genital herpes. *JAMA* 1982; 248: 1041–1049
75. Embil JA, Martin TR, Hansen NH et al: Group B beta haemolytic streptococci in the female genital tract: a study of four clinic populations. *Br J Obstet Gynaecol* 1978; 85: 783–786
76. Peevy KJ, Chalhub EG: Occult group B streptococcal infection: an important cause of intrauterine asphyxia. *Am J Obstet Gynecol* 1983; 146: 989–990
77. Baker CJ, Goroff DK, Alpert S et al: Vaginal colonization with group B *Streptococcus*: a study in college women. *J Infect Dis* 1977; 135: 392–397
78. Boyer KM, Gadzala CA, Kelly PD et al: Selective intrapartum chemoprophylaxis of neonatal group B streptococcal early-onset disease: 2. Predictive value of prenatal cultures. *J Infect Dis* 1983; 148: 802–809
79. McCormack WM, Almeida PC, Bailey PE et al: Sexual activity and vaginal colonization with genital mycoplasmas. *JAMA* 1972; 221: 1375–1377
80. McCormack WM, Braun P, Lee YH et al: The genital mycoplasmas. *N Engl J Med* 1973; 288: 78–89
81. Paavonen J, Miettinen A, Stevens CE et al: *Mycoplasma hominis* in nonspecific vaginitis. *Sex Transm Dis* 1983; 10 (suppl): 271–275
82. Blanco JD, Gibbs RS, Malherbe H et al: A controlled study of genital mycoplasmas in amniotic fluid from patients with intra-amniotic infection. *J Infect Dis* 1983; 147: 650–653
83. Kundsin RB, Driscoll SG, Monson RR et al: Association of *Ureaplasma urealyticum* in the placenta with perinatal morbidity and mortality. *N Engl J Med* 1984; 310: 941–945
84. Lamey JR, Eschenbach DA, Mitchell SH et al: Isolation of mycoplasmas and bacteria from the blood of postpartum women. *Am J Obstet Gynecol* 1982; 143: 104–112
85. Embree JE, Krause VW, Embil JA et al: Placental infection with *Mycoplasma hominis* and *Ureaplasma urealyticum*: clinical correlation. *Obstet Gynecol* 1980; 56: 475–481
86. Quinn PA, Shewchuk AB, Shuber J et al: Serologic evidence of *Ureaplasma urealyticum* infection in women with spontaneous pregnancy loss. *Am J Obstet Gynecol* 1983; 145: 245–250
87. Quinn PA, Shewchuk AB, Shuber J et al: Efficacy of antibiotic therapy in preventing spontaneous pregnancy loss among couples colonized with genital mycoplasmas. Ibid: 239–244
88. Lorenz RP, Appelbaum PC, Ward RM et al: Chorioamnionitis and possible neonatal infection associated with *Lactobacillus* species. *J Clin Microbiol* 1982; 16: 558–561